

Maritime Solutions Ballast Water Treatment System: A Shipboard Trial

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ABSTRACT

Ballast water management practices, voluntary for the most part now but expected to become mandatory in the near future, are largely based upon the seriously troubled practice of ballast water exchange with open ocean water. This approach puts many ships, their cargoes and, most importantly, the lives of their crews at risk due to the possible resulting loss of transverse stability and/or the consequences of longitudinal hull-girder failure. In the interest of providing a viable shipboard alternative to ballast water exchange, Maritime Solutions has lead the development of a two-stage system resulting in what is expected to be proven to be a safe, effective, practical, and cost effective solution. The MSI System is based upon the separation capability of the MST Microfugal Separator serving as the first stage and the UV technology of Aquionics, Inc. or, alternatively, the chemical biocide technology of Degussa AG and/or Vitamar, Inc. providing second stage treatment. MSI System testing is supported by grants made by the State of Maryland Port Administration and the National Oceanic and Atmospheric Administration (NOAA). Working in cooperation with the Center for Environmental Science, part of the University of Maryland, testing will take place aboard the "CAPE MAY", a 39,000 DWT MARAD cargo vessel, spring 2001. The U.S. Coast Guard will observe the program and the Chamber of Shipping of America will provide peer review.

Current IMO prescribed ballast water management practices, voluntary for the most part now but expected to become mandatory in the near future, are largely based upon the seriously troubled practice of ballast exchange with open ocean water. Ballast water exchange at sea puts many ships, their cargoes and, most importantly, the lives of their crews at risk due to the possible changes in transverse stability and/or longitudinal hull-girder loading. Beyond this, ballast water exchange has, with little exception, been variously determined to achieve a level of only 65 % to 95 % effectiveness in the exchange of the original ballast water; the actual result being dependent on ship type (tanker, bulk carrier, containership, etc.), the specific design of a particular vessel, and its trade route or voyage pattern. In fact, the level of effectiveness of ballast water exchange is 0 % when it is not practiced (i.e., whenever the Master determines that 'conditions' do not allow it to be performed). At the same time, only a fraction of the sediment contained in the original ballast water is eliminated, leaving a refuge and an active breeding ground for many marine organisms. It is, as a result, abundantly clear that higher-level technology needs to be employed to assure shipboard safety, to reduce sediment loading in ballast water, and to provide for a higher level of effectiveness in the mitigation of biological invasions.

GENERAL INFORMATION

In the interest of offering a viable shipboard alternative to ballast water exchange, Maritime Solutions, Inc. has lead the development of a two-stage system as recommended by The Shipping Study (Carlton et al. 1995), wherein it was clearly predicted that a multi-stage system would be necessary to

effectively mitigate against sediment and organism introduction by ballast waters. The approach taken by Maritime Solutions also conforms with the conclusions reported by the National Research Council (1996) in that it couples state-of-the-art separator technology with advanced UV or, alternatively, chemical biocide technology resulting in what is expected

to be a safe, effective, practical, and cost effective solution to the ballast water problem.

The resulting 'Maritime Solutions Ballast Water Treatment System' (MSI System), patent pending, is based upon the separation technology of Maritime Solutions Technology, Inc. (MST), serving as the first stage and the UV technology of Aquionics, Inc. or, alternatively, the chemical biocide technology of Degussa AG and/or Vitamar, Inc. providing second stage treatment. The two-stage MSI System offers the promise of superior organism elimination, increased silt and sediment reduction (> 90%), and flow rates to meet shipboard requirements (e.g., 300 - 20,000 m³h⁻¹); all within a compact, crew friendly and energy efficient installation. Maritime Solutions is currently involved in a rigorous program of system engineering and independent shipboard system testing with oversight from the U.S. Coast Guard.

The MSI System testing is supported by grants made by the State of Maryland Port Administration (MPA) and the National Oceanic and Atmospheric Administration (NOAA). Working in cooperation with the Center for Environmental Science, part of the University of Maryland, Maritime Solutions has additionally won the support of the U.S. Maritime Administration (MARAD) that will allow the testing to take place aboard the *Cape May*, a ship of the U.S. Ready Reserve Force. The former Lykes Lines SEABEE vessel of 39,000 tons dead weight (DWT), now berthed in the Port of Baltimore, Maryland and managed by Interocean Ugland Management, will allow for realistic shipboard testing of the MSI System in treating water taken from Baltimore Harbor and the Chesapeake Bay. Additionally, the U.S. Coast Guard, with support from the Volpe National Transportation Center, will audit the test program. The Center for Environmental Science serves as the independent testing body and, as a result, is providing the principal investigators to oversee the testing program. The Chamber of Shipping of America is providing for 'peer review' via a committee of maritime industry experts.

The MPA and NOAA grants coupled with the financial support and in-kind contributions of the other program participants and MSI System component suppliers have made for a public/private sector initiative that has a total value approaching One Million (\$1,000,000) U.S. Dollars.

The MSI System will utilize the proprietary MST Microfugal Model MSX-1500

Separator to separate the components of the influent ballast water in the primary treatment stage. As a primary treatment, the MST Microfugal Model MSX-1500 Separator is intended to remove silt and sediments and certain large organisms from the influent ballast water and then immediately return these materials back to the source waters in a small fraction of the water stream. The remaining 'clean' water stream is then to be treated by UV or, alternatively, by chemical biocide in a secondary system stage. The primary treatment stage will be assessed as to its value in removing sediments from ballast-bound water, as well as its effect on the efficacy of the secondary treatments. Following treatment, the 'cleaned' and disinfected ballast water will be transferred via the vessel's ballast pump to the ballast tanks.

Maritime Solutions is working with leaders in the fields of UV testing and biocide toxicity testing who will participate in this project. New high intensity UV systems have been developed which promise to increase treatment effectiveness against a broader spectrum of organisms and, at the same time, decrease necessary exposure time, which is critical in the high flow-rate systems needed for shipboard ballast water treatment systems. The use of two new chemical biocides will also be tested; both proprietary compounds having short (hours) half-lives. Short half-life is essential because it reduces the required period of shipboard holding and the potential environmental problem of introducing these chemical compounds into the environment when the treated ballast water is finally discharged. Thus, Maritime Solutions is coupling the MST Microfugal Separator technology with both recommended approaches to secondary treatment in order to determine and verify the increased level of overall treatment effectiveness that results from the removal of larger organisms as well as the removal of silt and sediment particulates that would otherwise shield organisms from UV treatment or that would interfere with chemical biocide treatment necessitating an increase in chemical dosage.

By removing particles more dense than water (e.g., clays, silt, and detrital material) from the influent water, the first stage MST Microfugal Separator will make ultraviolet treatment in the second stage more effective because of the reduction in 'shadow' produced by these particles. Similarly, particulate removal will assist when chemical biocides are used as the secondary means of treatment. Adsorption of

compounds into clays and humic materials (detritus) occurs in many applications (Khan and Dupont 1987; Morillo et al. 1992; Piccolo et al. 1998; Undabeytia et al. 2000). Adsorption percentages of some herbicides can be as high as 42% on clay alone and much higher on organo-clay complexes (binding coefficients as high as $1500 \text{ k(M}^{-1}\text{)}$, Undabeytia et al. 2000). Thus, clays, silts, and detritus have a high potential to force an increased application of chemical biocides in order to achieve effective treatment of ballast water. In addition, the high-binding capacity of these sediments for biocides suggests that in cases of sediment build-up in ballast tanks, a sediment refuge may be established. Adsorption by overlying sediments may produce a biocide barrier to organisms including bacteria and viruses, cysts of dinoflagellates, the resting stages of crustaceans, and burrowing invertebrates.

Maritime Solutions Technology, Inc. – MST Microfugal Separator - The proprietary MST Microfugal Model MSX-1500 Separator is a continuous flow machine that is designed to generate high centrifugal forces (i.e., estimated at 1,300 “g” for operating conditions in this test, Sigma Design Company, L.L.C.) capable of separating particles and organisms of different specific gravities including those of small micron size (i.e., estimated $< 5 \text{ }\mu\text{m}$, Sigma Design Company, L.L.C.) from the liquid stream at extremely high flow rates per unit size of separator. As the liquid stream passes through the MST Microfugal Separator particle separation is accomplished by a single rotating impeller of proprietary design that is contained within a Sterling Fluid Systems (USA), Inc. designed and fabricated housing that is coupled to a static separation chamber. In liquid/solid mixture separation, the separator’s centrifugal forces cause the denser particle components to gravitate to the outside of the liquid stream. The liquid stream is divided into separate fractions as a function of relative density as it passes through the separation chamber. The various liquid and solid fractions are ultimately separated at the discharge end of the chamber where they pass through separate collection ports with the ‘clean’ water passing on to secondary treatment.

Aquionics – In-Line UV Treatment System - The Aquionics In-Line UV treatment system has been selected for incorporation in the MSI System because of its superior design, quality of construction and proven ability to provide proper disinfection, even to poor quality liquid streams. In order to properly disinfect,

UV germicidal energy must pass through all of the fluid that requires treatment. If even 1% of the liquid stream goes untreated there will be a dramatic reduction in overall effectiveness. A standard design UV chamber will not be effective in treating water with poor UV transmission because of the hydrodynamic complexities affecting the application of uniform UV treatment. The Aquionics In-Line chamber, with its unique design, was developed to address just this problem.

The Aquionics In-Line chamber system contains multiple lamps (specific number determined by treatment and flow rate requirements) installed perpendicular to the liquid flow. The lamps are situated in such an arrangement that all of the liquid (in this case influent ballast water) is forced to pass within close tolerances to the surface of the high intensity, medium pressure UV arc tube lamps thereby eliminating untreated ‘dead legs’ of water. In addition, the Aquionics In-Line UV systems are self-cleaning and monitoring and can treat flows ranging from 50-13,000 gpm (i.e., $11\text{--}2,955 \text{ m}^3\text{h}^{-1}$). Minimal annual maintenance is required.

Degussa AG – PERACLEAN® OCEAN - PERACLEAN® OCEAN is a special biocide formulation based on peroxy acetic acid for ballast water treatment. It has excellent biocidal, virucidal and fungicidal properties at very low concentrations ($<100 \text{ ppm}$) as well as good effectiveness on phytoplankton, zooplankton and other species found in the ballast water of ships. PERACLEAN® OCEAN is effective over a wide range of pH and temperatures. It is also readily biodegradable according to OECD test guidelines. Residual PERACLEAN® OCEAN in ballast water decomposes to water, acetic acid (e.g., vinegar) and oxygen. The half-life is in the range from 10 minutes to 24 hours depending on pH, salinity and temperature.

PERACLEAN® OCEAN is commercially available in 220 l. drums, 1 m^3 IBCs or in bulk containers. PERACLEAN® OCEAN itself has a shelf life of $> 1 \text{ year}$ ($< 10\%$ loss in activity). Analytical methods to determine PERACLEAN® OCEAN in ballast water have been developed. Test strips for quick semi-quantitative analysis of residual PERACLEAN® OCEAN in ballast water are also available.

Vitamar, Inc. – SEAKLEEN® - SEAKLEEN® represents another of a new class of environmentally friendly natural product biocides. It is a non-halogenated nutrice of mammalian/microbial origin.

Fluid Imaging Technologies, Inc. – FlowCAM®
On board monitoring of the MSI System is to be performed by Fluid Imaging Technologies, Inc.'s FlowCAM®; an imaging flow cytometer that monitors liquids for the presence of particles from 5 µm to 1000 µm in discrete samples or from continuous sources. Continuous sources such as ballast water can be monitored for extended periods of time with only a limited weekly maintenance requirement. The FlowCAM® measures in-vitro or discrete samples with a flow rate of 10 ml·min⁻¹. Each particle is automatically imaged and measured and the data directly stored to disk. FlowCAM®'s image recognition software processes all particle images to determine particle types present. The instrument measures several particle properties, including fluorescence, light scatter, size and time of particle passage. These measurements and the image recognition software make it possible to determine how many of the different organisms are present at any given time. Because the FlowCAM® provides continuous, real-time particle data, it will be useful for assuring proper operation of the particle separator. It will also monitor the effectiveness of these treatments as the water-borne organisms die off in the ballast tanks.

Program Management, Participation and Support - Maritime Solutions is managing this ballast water treatment system program and has provided for project planning as well as specialized personnel services in order to assure program success. In addition to its own staff, Maritime Solutions has the benefit of the active involvement of Unitor AS, Oslo, Norway; Aquionics, Inc., a Halma Group Company, Erlanger, Kentucky; Degussa-Hüls Aktiengesellschaft, Frankfurt, Germany; Vitamar, Inc., Memphis, Tennessee; and Fluid Imaging Technologies, East Boothbay, Maine. Beyond this, Maritime Solutions has the benefit of the involvement of a number of leading marine and equipment system consultants and subject matter experts who have committed to participate in this development program. These include Sigma Design Company, L.L.C., Springfield, New Jersey; Martin, Ottaway, van Hemmen & Dolan, Inc., Red Bank, New Jersey; and Environmental Research Services, Solomons, Maryland.

Project Schedule

Winter 2000-2001

Computer fluid dynamics modeling completed by Sigma Design Company, L.L.C. to confirm the optimal design characteristics for the Separator

Complete engineering design to integrate the test system with the ballast system on board the "CAPE MAY"

Develop dosing systems for the chemical biocides Peraclean Ocean® and Seakleen®

Contract for the fabrication/assembly of the complete MSI System

Establish locations on board the "CAPE MAY" or locally to process samples and conduct latent effects analysis

Spring - Summer 2001

Complete installation of the MSI System aboard the "CAPE MAY"

Conduct preliminary testing of each system component

Conduct intensive system test and sampling

Summer - Fall 2001

Data analysis and report completion

Submit reports to NOAA, MPA, MARAD, U.S. Coast Guard and IMO

Winter – 2001-2002

Obtain the required governmental (i.e., U.S. Coast Guard and/or IMO) approval of the MSI System as an alternative to 'ballast water exchange'

Install MSI Systems aboard trading merchant vessels

The prototype MSI System consists of an MST Microfugal Model MSX-1500 Separator working in series with an Aquionics UV treatment system capable of delivering 100 mWs·cm⁻² or, alternatively, in series with two independent chemical biocide dosing units. The entire system will be mounted in the machinery space of the "CAPE MAY" and connected to the ship's ballast water system. The installation will be made in such manner that collection of physical and biological samples can be made before and after all treatment combinations.

Given the above, important sampling design and procedural issues include: Development of an installation design and sample collection process that provides for unbiased estimates of effectiveness, sufficient information to assess effectiveness over a range of conditions, and a standardized manner that can be repeated in subsequent tests.

Determination of what and how many specific primary and secondary treatments should be applied during the testing program. That is, what will be the operating parameters for the primary treatment in terms of flow rate, characteristics of UV light (intensity and special composition) and, alternatively, concentration of chemical biocide as a secondary treatment.

Determination of the environmental variables to be tested in the on board assessment. That is, how many and what experimental combinations of environmental (and biological) conditions are to be tested in the experiment?

Selection of the environmental variables to be quantified, organisms to be assessed (and how this will be conducted) for effectiveness of each ballast water treatment combination, as well as procedures for sampling, processing and statistically analyzing the samples taken during the testing process. Provision to be made for determination of latent effects.

Sampling Design

Treatment Combinations (Total = 12)

Control (by-pass all treatments)

Sample at Ports A and C.

Separation Only:

Sample at Ports A, B₁, B₂, and C.

Separation + UV:

Sample at Ports A, and C.

Separation + PERACLEAN[®] OCEAN (2):

Sample at Ports A, and C.

Separation + SEAKLEEN[®] (2):

Sample at Ports A, and C.

UV alone:

Sample at Ports A, and C.

PERACLEAN[®] OCEAN alone (2):

Sample at Ports A, and C.

SEAKLEEN[®] alone (2):

Sample at Ports A, and C.

There are a total of twelve treatments owing to the fact that two concentrations of each of PERACLEAN[®] OCEAN and SEAKLEEN[®] will be tested.

Sample Collection - During each trial, water will be collected simultaneously at each of the sampling ports needed for the specific treatment using identical taps (to prevent different effects of tap aperture design on organism survivorship, especially zooplankton) and collecting similar volumes of water (~400 liters = ~100 gallons) over the duration of at least one minute. The value of this long collection time is to maximize the likelihood that the same body of water is sampled at all ports even though

there is travel time between ports. That is, integrated samples will be taken over sufficient time that differences in water masses sampled due to travel time will be minimized. These water samples will be collected in ~600-liter polyethylene tanks. In addition, the installation design calls for samples to be collected in both polyethylene tanks and in the ship's ballast water tanks. While taking samples from ballast tanks has its challenges, it is deemed necessary for this test program. Finally, provision will be made that all control and treated water passes through the same ballast pump.

Sampling Effort

An extensive sampling effort consisting of the following treatments and trials:

12 treatment combinations

3 trials per treatment

36 total trials

The purpose of sampling ports B₁ and B₂ off of the MST Microfugal Separator is the collection of samples of the water stream that will be discharged back into the Chesapeake Bay. Some organisms will be entrained in this discharged water, as will a majority of the sediment. The sediment load and organism abundance in these effluent waters will then be sampled and analyzed. Alternatively, determination of the fraction of these variables removed by the primary treatment could be made when samples are collected from Ports A and C (i.e., by subtraction). The disadvantage in the alternative approach is that there will be no explicit determination that the sum of the discharge concentrations equals the intake concentration of any variable. The advantage is a reduction in the sampling effort per trial and thus an opportunity to increase the number of trials (replicates). The approach to be taken is a series of pilot trials to quantify the sediment load and organism abundance from all ports (i.e., A, B₁, B₂ and C) and confirm that all material entering the intake can be accounted for. If, as expected, this approach is confirmed to be appropriate, samples will be taken only from Ports A and C reducing the sampling effort per trial and allowing for an increase in the number of trials per treatment.

Each of the single or combination treatments is to be repeated three times. Although these trials can not be called true replicates (which would be impossible in a field trial of this nature) they will provide an estimate of the degree of repeatability of the tests under

conditions that may differ from day to day or from week to week (depending on the periodicity of the repeated tests). Each test or group of tests will be accompanied by a 'control' in which ballasting will proceed without any of the treatments referred to above. An effort will be made to conduct a control test as close in time as possible to the respective treatment. Ideally, this will best be performed at the ballast water intake as part of a single ballasting operation, with the equipment turned off (immediately before or after a treatment phase). The aim will also be to sample different parts of the ballast water system of the vessel following a treatment cycle. While this may not be possible to do for all of the aforementioned treatments, an attempt will be made to sample as many ballast access areas as possible.

Replication - Three trials will be conducted for each treatment alternative. For example, if a comparison is being made of chlorophyll *a* concentration in samples between sampling ports and three trials are made, three estimates of the proportional decline in chlorophyll *a* will result. These three estimates can be compared with estimates from other treatments using ANOVA (or the nonparametric equivalent, Kruskal-Wallis test).

Research Methods for all of the treatments and sampling described above, a suite of physical and biological observations designed to specifically test the effectiveness of each treatment will be made.

Environmental Conditions - Most of the environmental variables in this shipboard test cannot, of course, be controlled. However an effort will be made to minimize the variability of these variables within the testing period, as well as to determine the effectiveness of the treatments across a range of sediment loading conditions. Thus, in each trial, when applicable, the following will be quantified:

Temperature

Salinity

pH

Sediment load (total, organic and inorganic fraction, and particle size distribution)

Flow rate (specifically at the intake and the two outlets of the MST Microfugal Model MSX-1500 Separator)

Light transmission (absorbance, to quantify exposure rates for UV testing)

Concentration of chemical biocides applied and at 24 and 48 hours after application

Suspended Sediments - For treatments involving the MST Microfugal Separator, it will be important to determine performance in removing a range of suspended sediments and, when the separator is not being used, it will be important to know the sediment-loading rate to assess the impact on the secondary treatment systems. The sediment loading of estuarine waters is expected to range from an ambient mid-water level of $<0.1 \text{ g l}^{-1}$ to as much as 50 g l^{-1} in heavily loaded (turbidity maxima) regions (Li 1999). Separatory performance will be determined by making a series of measurements of ballast-bound water from the intake and exit streams of the separator. These measurements will include determination of (1) total sediment mass at the sampling ports, (2) light transmission characteristics (3) particle size fractions, and (4) of ash-free dry mass.

Total solids per unit volume will be determined by collecting two depth-integrated sub-samples from the collection tanks, and then passing each through oven-dried and pre-weighed $0.4 \mu\text{m}$ Nucleopore filters. The volume of each sub-sample processed will depend upon the sediment load. Sub-samples will be dried at 60°C for 24 hours, cooled in a desiccator and then weighed to estimate total sediment load. An alternative approach when sediment loads are high will be to place volumes of the sub-sample into pre-weighed ceramic crucibles, evaporate contained water in drying ovens (60°C) and then reweigh the crucibles. Ash-free dry mass (AFDM, i.e., the inorganic fraction of the sediment) will be estimated by ashing the dried sub-samples at 550°C for 24 hours and then reweighing the residue. For sub-samples processed through membrane filters, appropriate standards (blank filters) will be processed in order to remove the contribution of the filters to the residue mass. This process will provide data on percent organic matter in samples.

Particle size distribution will be quantified from at least 500 particles in samples taken from each sampling port under the two sediment loads (i.e., ambient mid-water, and mechanically elevated condition). Samples will be filtered through blank $0.4 \mu\text{m}$ Nucleopore filters and then later dispersed by ultrasonic treatment for 2 minutes (Eisma 1986; Li et al. 1999). Particular profiles from these samples will be generated using a Model IIA flow cytometer. This will produce comparative size

data that can be calibrated with the addition of particles of known size. Profiles will be checked with a 'Multisizer' particle counter that will supply a frequency distribution over the whole particulate size range.

It is anticipated that organisms will form part of the fraction 'filtered' by the MST Microfugal Separator. As indicated above, this fractional removal will be quantified in preliminary trials with the expectation that the numbers in the trials can be determined through density differences from Ports A and C.

Bacteria

Bacterial viability will be assessed using the two-color fluorescence assay, namely the live/dead Bac Light TM bacterial viability kit for microscopy (Molecular Probes, Eugene Oregon). The live/dead assay uses a Syto green fluorescence nucleic acid stain and the red fluorescence nucleic acid stain propidium iodide. These strains differ both in their spectral characteristics and their ability to penetrate healthy cells. Samples of the ballast water pre- and post-treatments will be filtered onto black, 0.2- μ m Nucleopore filters stained with the two-component mixture and the red vs. green fluorescence of bacterial cells counted by epifluorescence microscopy using appropriate wavelength filters.

From the 36 trials planned for this study we will be collecting at two ports per trial and analyzing certain viability initially and at 24 and 48 hours post-test to determine the latent mortality effects of treatment. Thus we will be collecting a total of 216 samples. During incubation periods, samples will be aerated in the dark at ambient ballast water temperature.

Phytoplankton

Phytoplankton biomass and viability are important components in the assessment of both primary and secondary treatments. As most phytoplankton species expected to be seen in Baltimore Harbor waters are $<10 \mu\text{m}$ and have specific gravity near that of ambient water the separatory ability of the MST Microfugal Separator is uncertain in this regard and secondary treatment will be an important component of the overall treatment strategy. Measurements will, therefore, be directed towards the assessment of both phytoplankton biomass (chlorophyll *a*) and the capacity for regrowth after treatment.

Extractable chlorophyll *a* fluorescence determination will be made on all treated and untreated ballast water samples. Whole water samples will be taken from the collection tanks and particulates extracted using glass fiber filters which will be used to determine concentrations of chlorophyll *a* over time in pre- and post-treatment samples (sampling ports A and C). The standard operating procedures to be followed will be those outlined in Standard Methods for the Examination of Water and Wastewater, 20th Edition (1998) under sections 10-20 and 10-21.

Biological Examination: The testing instrument employed will consist of a Hitachi F4500 scanning spectrofluorimeter with a dedicated computer controller. Excitation will be set at 430nm with a slit width of 10nm. Emission wavelength will be fixed at 633nm with a 10nm slit width. For extractable chlorophyll *a*, standard operating procedures outlined in Standard Methods for the Examination of Water and Wastewater, 20th Edition (1998) under sections 10-20 and 10-21, "Biological Examination" will be followed with the exception that the extraction solvent will be ethanol, which has proven to be more efficient in extracting chlorophyll *a* from glass fiber filters at room temperature in the dark for 3 hours. A total of 216 samples will be processed with each technique.

Latent Effects: Water samples will be aerated and illuminated under fluorescent lights on site for a 48-hour period to examine the capacity for cell doubling based on repeated measurement of chlorophyll *a* fluorescence. This capacity for regrowth will be the unit of measure to determine latent effects of each treatment and follows the standard operating procedures as outlined in Standard Methods for the Examination of Water and Wastewater (1998). After exposure to light for 24 and 48 hours, under aerated conditions and ambient temperature, each sample of phytoplankton will be resampled to estimate chlorophyll *a* concentration.

Cell Counts: To quantify phytoplankton abundance (cell counts) in order to compare with chlorophyll *a* concentration, subsamples of collected water will be taken and examined under the microscope (i.e., Olympus BH2) for cell counts and taxonomic identification. Chlorophyll *a* concentration is species and time dependent, as well as being dependent upon cell

biovolume. Taxonomic identification and cell counts of phytoplankton will be made on the following subset of samples:

Separation and UV, PERACLEAN® OCEAN or SEAKLEEN® (5 treatments)

Ports A and C

Replication: 3

Timed samples: initial, 24 and 48 hour

Total samples to be processed: 90

Zooplankton

Four to six of the most common zooplankton taxa will be identified in the local water at the time of the trials, and abundance and survivorship of these taxa will be quantified. At least one rotifer, two crustaceans (an adult copepod (*Eurytemora affinis*) and a naupliar stage), and hopefully a mollusk larva (oyster).

To assure the proper collection of samples from the collection tanks, the water in the tanks will be mixed thoroughly immediately after collection and three or more depth integrated samples (using a 10-cm diameter clear polycarbonate cylinder) will be taken from the center tank, pooled (total volume recorded) and then concentrated through a 20 µm mesh Nitex filter chamber that is submerged in water to minimize handling damage to zooplankton. The sample will be transferred into 2- 4 liter holding chambers of known volume. Subsamples of organisms in the holding chamber will be collected with small depth-integrated samplers. Density, as well as numbers alive, will be determined until at least 50 of each taxon are counted. Organisms in holding chambers will be exposed to aerated water at ambient temperature and will be kept in total darkness until it is time to quantify survivorship through the latency periods. As with the phytoplankton and bacteria sampling a total of 216 samples will be collected.

Latent Effects: Data for SEAKLEEN® from Vitamar through Drs. Wright and Dawson (University of Maryland Chesapeake Environmental Laboratory) and for PERACLEAN® OCEAN from Degussa AG suggest that 24 hours should be sufficient time for mortality to approach 100% and for the biocides likely be degraded at the concentrations to be used. However, because the sediment loading will reduce chemical biocide and UV effectiveness to some extent, survivorship of zooplankton will be determined at both 24 hours and 48 hours after each trial.

Counting: As indicated above, the volume of water collected in the sample tanks, the subsampled water volume, and the concentrated holding chamber volume will be recorded, along with the volume sampled in the holding chamber in order to back calculate the density of zooplankton taxon in the water from the sampling ports. Each time counts and survivorship are determined an attempt will be made to locate at least 50 organisms of each taxon. Because density and survivorship will be recorded, any decline in the density (i.e., decomposition of dead organisms) in any holding tank can be determined.

Data Analysis

Data will be analyzed using desktop software such as Microsoft Excel, QIAGEN and system software such as Statistics and Analysis System (SAS). Efficiency of treatments will be determined first using a paired t-test to determine the statistical difference ($p < 0.05$) between treatments and controls for each trial. Where there is a statistical difference, the average percent efficiency for each treatment (relative to the control) will be determined for that trial. These average efficiencies will be used to determine a mean efficiency and standard deviations across trials.

Oversight

The U.S. Coast Guard has been invited to conduct a review of the test protocol before it is finalized. Beyond this, the U.S. Coast Guard and the Volpe National Transportation Laboratory have been invited to monitor the testing program, including matters of sampling and data analysis.

CONCLUSION

After independent testing and reporting on performance has been completed, Maritime Solutions believes that the MSI System will be recognized as being superior to any other alternative to ballast exchange proposed to date for the following reasons:

The system is completely scaleable and can, as a result, produce ballast water flow rates equal to the loading rates required by all merchant and naval vessels.

The system provides an economic benefit to ship owners/operators due to its removal of silt and sediment from the ballast water intake stream, obviating the need for periodic and expensive tank clean-out and

insuring, all the while, the maximum cargo carrying capacity of the vessel.

The system's 'secondary' treatment stage, UV or chemical biocide subject to throughput capacity requirement, is extremely effective and safe for both the crew and the environment. Ballast water 'residence' time associated with effective 'secondary' treatment is significantly reduced due to the system's removal of entrained silt and sediments and does not, as a result, hinder the ballasting process and the vessel's time schedule.

The compact size and energy efficiency of the complete two-stage system allows for easy, cost effective, retrofit or installation and operation aboard both existing vessels and new building tonnage.

The environmental benefits accruing from the ship's ability to utilize the system at the time of every ballasting, with no subsequent impact or slowdown on other vessel activities or operations.

The system has no crew, vessel, or cargo related safety (stability and trim, longitudinal hull strength, etc.) issues as are associated with the current practice of ballast water exchange.

The system will be virtually automatic, requiring minimal crew training and operating instructions. Owing to its design simplicity and quality of construction, the system is virtually clog-free and requires limited maintenance.

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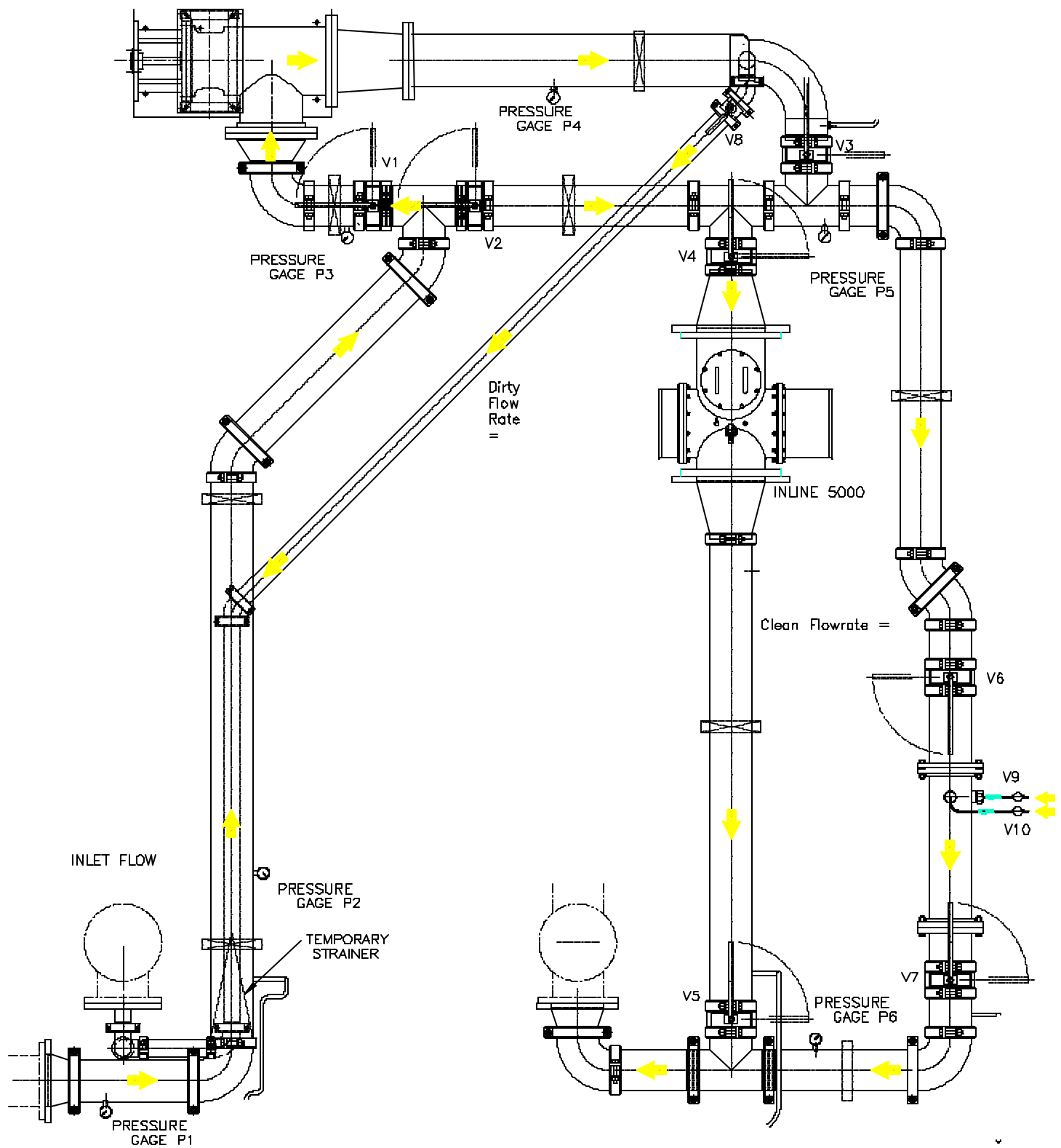
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**MSI Ballast Water Treatment System
S.S. CAPE MAY test installation**